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Phyllidiella pustulosa

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A report on species of phyllidiid and polycerid nudibranch including two species new to Korea

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During a systematic study on Korean nudibranchs, two phyllidiid and two polycerid species were collected and identified with examination of their external morphological characters. As a result of the study, two phyllidiid species, *Phyllidia ocellata* Cuvier, 1804 and *Phyllidiella pustulosa* (Cuvier, 1804) were re-described since there was no descriptions of these species with Korean specimens. And two polycerid species, *Thecacera pennigera* (Montagu, 1815) and *Triopha catalinae* (Cooper, 1863) were described as new to Korean fauna. Diagnostic characters of these species are described with illustrations in this study. DNA barcode data from three species examined were also provided for a supplement to morphological identifications.

Keywords: Korea, Nudibranchia, *Phyllidia ocellata*, *Phyllidiella pustulosa*, Phyllidiidae, Polyceridae, *Thecacera pennigera*, *Triopha catalinae*

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INTRODUCTION

Nudibranchs comprising more than 6,000 species worldwide are distinguished by missing a shell in the adult stage. Species included in the family Phyllidiidae having no primary gill and comprising hard tubercles in dorsal part of body can be easily separated from the species of related families (Gosliner *et al.*, 2008). Within the family, species are distinguished by characteristic color variation of body and spacing of the dorsal tubercles. The family Polyceridae is distinguished by having velar tentacles along anterior part of body, and extrabranchial and lateral appendages (Gosliner *et al.*, 2008).

Until now 8 species in families Phyllidiidae and Polyceridae have been recorded from Korean waters (Choe and Lee, 1997; Lee and Min, 2002; Choi, 2003): *Phyllidia ocellata* Cuvier, 1804; *Phyllidia picta* Pruvot-Fol, 1957; *Phyllidiella cooraburrama* Brunkhorst, 1993; *Phyllidiella pustulosa* (Cuvier, 1804) in the family Phyllidiidae; *Plocamopherus tilesii* Bergh, 1877; *Tambja amakusana* Baba, 1987; *Tambja sagamiana* (Baba, 1955); *Tambja verconis* (Basedow and Hedley, 1905) in the family Polyceridae. There was no taxonomic report with descriptions on species included in these families from Korean waters.

In this study, we reported brief re-descriptions with figures of two species in the family Phyllidiidae, *Phyllidia ocellata* Cuvier, 1804 and *Phyllidiella pustulosa* (Cuvier, 1804) and two species in the Polyceridae, *Thecacera pennigera* (Montagu, 1815) and *Triopha catalinae* (Cooper, 1863) as new to Korea with presentation of diagnoses and illustrations. In addition, mitochondrial COI DNA sequence data of three species examined were presented for an aid of species identification.

MATERIALS AND METHODS

Materials were collected by scuba diving in the subtidal zone of the Korean coast from June 2011 to October 2012. The collected specimens were narcotized with a 10% MgCl₂ solution, and fixed in 10% neutral buffered formalin or 97% ethanol to preserve. The specimens were examined with a stereoscopic microscope (Olympus SZ-61 with FuzhouTucsen TCA-3). Body lengths of most specimens were measured from the middle points of the rhinophores to the tip of posterior end by using ocular micrometers. Examined specimens were deposited at the National Institute of Biological Resources (NIBR), Incheon, Korea and Sangmyung University, Seoul, Korea. NIBR specimen numbers are recorded for the specimens.

For molecular identification, genomic DNA was extracted from isolated tissues. Mitochondrial COI gene was amplified (500 bp) using a universal primer set (LCO1490, HCO2198; Former *et al.*, 1994). PCR products were purified and analyzed by DNA sequencing. Each of the primers was removed and open reading frame was obtained as sequences translated into protein to avoid numts. GenBank reference sequences have been searched to the resulting sequences using BLAST.

SYSTEMATIC ACCOUNTS

Phylum Mollusca Linnaeus, 1758
Class Gastropoda Cuvier, 1817
Order Nudibranchia Cuvier, 1817
Family Phyllidiidae Rafinesque, 1814

Diagnosis. Elongate-ovate shape. Retractable lamellate rhinophores. Primary gill absent. Secondary gill flat and triangular in shape. Mantle skirt covers the foot. Tough body and generally possessed hard dorsal tubercles. Radula or jaws absent (Brunckhorst, 1993).

Genus *Phyllidia* Cuvier, 1797

Phyllidia ocellata Cuvier, 1804 (Fig. 1)

Phyllidia ocellata Cuvier, 1804: 269, pl. a, fig. 7 (cited from Yonow, 1996). Brunckhorst, 1993: 35-37, figs 25D-E, pls. 2D-H, 3A; Yonow, 1996: 485-487, fig. 1, tab. 1; Choe and Lee, 1997: 38, tab. 1; Okutani, 2000: 795, fig. 3; Lee and Min, 2002: 145; Choi, 2003: 47, pl. 5; Debelius and Kuiter, 2007: 265; Gosliner *et al.*, 2008: 287.

Phyllidiopsis carinata Eliot, 1910: 435-436, pl. 25, figs. 8, 9, 12 (cited from Yonow, 1996).

Phyllidia multituberculata Boettger, 1918: 129-130, pl. 8, fig. 4a-c (cited from Brunckhorst, 1993); Yonow, 1996: 487-490, figs. 2A-E, 3A-G, 4B, tab. 1.

Phyllidia tuberculata Baba, 1930: 117-118, pl. 4, fig. 1a-d (non *Phyllidia tuberculata* Risbec, 1928) (cited from Brunckhorst, 1993).

Phyllidia japonica Baba, 1937: 310 (cited from Brunckhorst, 1993); Baba, 1949: 72, 157, pl. 29, fig. 108; Lim and Chou, 1970: 134, pl. 16c (cited from Yonow, 1996).

Phyllidia baccata Pruvot-Fol, 1957: 111-113, figs. 13-21, pl. 1b (cited from Brunckhorst, 1993).

Phyllidia ocellata undula Yonow, 1986: 1411-1413, figs. 5, 12a, b (cited from Brunckhorst, 1993).

Material examined. 1 individual, Jeju-do, Seogwipo-si, Seogwi-dong. 19 Feb 2012; 1 individual (KOSPIV0000 164403), Jeju-do, Seogwipo-si, Seogwi-dong. 13 Oct

2013; 1 individual, Jeju-do, Seogwipo-si, Seogwi-dong. 14 Oct 2013.

Diagnosis. Body ovate and elongate (length: 42-63 mm, width: 26-40 mm). Rhinophores lamellate and dark yellow in color (Fig. 1A). Anus open with yellow border. Anus papilla white (Fig. 1B). Back ground color brightly yellow. Dorsum several various sizes of patches of black rings bordered with white, rounded or conical tubercles scattered. Median dorsal tubercles appear to line up longitudinally. Tubercles usually decrease in size from top to edge of mantle (Fig. 1C). Sole of foot gray, divided anteriorly (Fig. 1D). Oral tentacles finger like in shape, rounded ends (Fig. 1E).

Distribution. Korea, Southern Japan, Thailand, Philippines, Indo-west Pacific, Indonesia, Australia, South Africa, Tanzania, Madagascar, Mauritius, Seychelles, Red Sea to Maldives, Fiji, Solomon Island, Papua New Guinea, Vanuatu, Guam to Marshall Island.

DNA barcode. COI sequences of the first mentioned specimen in the "Material examined" are as follows: 5'-CGATCATTTTTATAATGTTATTGTAACAGCTCATGCTTTTGTAATAATTTTCTTTTGGTAATACCTTTAATAATTGGGGGGTTCGGAATTGAATGGTCCATTACTAATTGGTGCTCCAGACATAAGATTTCCCCGAATAAATAATATAAGTTTTTGGTTACTACCACCATCTTTTATTTTATTATTATGCTCAACTTAAATAGAAGGTGGAGCTGGAACAGGGTGAAGTGTATATCCCCCATTATCTGGACCAATAGGACATGTGGGACTTCTGTTGATTAGTAATTTTTTCTTTACACTTAGCTGGAGCCTCTTCTTCTAGGGGC AATTAATTTTATTACTACTATCTTCAATATACGTTCACCTGCTATAACAATGGAACGTTTAAGATTA TTTGTTTGGTCAGTTTTAGTGACAGCTTTTCTTT TACTTCTTTCTTTACCTGTTTTAGCAGGGGCTAT TACTATACTTTTAACGGATCGAAATTTTAACA-3'.

According to BLAST search to GenBank, this sequence matches 88% with *Phyllidia elegans* (AJ223276). There is no COI sequence information for *P. ocellata* in the GenBank. Therefore more COI records from different localities of the species are needed for correct molecular identification.

Remarks. *Phyllidia ocellata* is known as variety of color pattern, even from a single locality (Gosliner *et al.*, 2008). In Korea, however, all of specimens examined in this study and previous study (Choi, 2003) are shown same dorsal patterns with bright yellow background and 7-8 black thick rings and several patches.

Genus *Phyllidiella* Bergh, 1869

Phyllidiella pustulosa (Cuvier, 1804) (Fig. 2)

Phyllidia pustulosa Cuvier, 1804: 268, pl. a, fig. 8 (cited from Brunckhorst, 1993); Baba, 1949: 18, 71, 156, pl. 29.

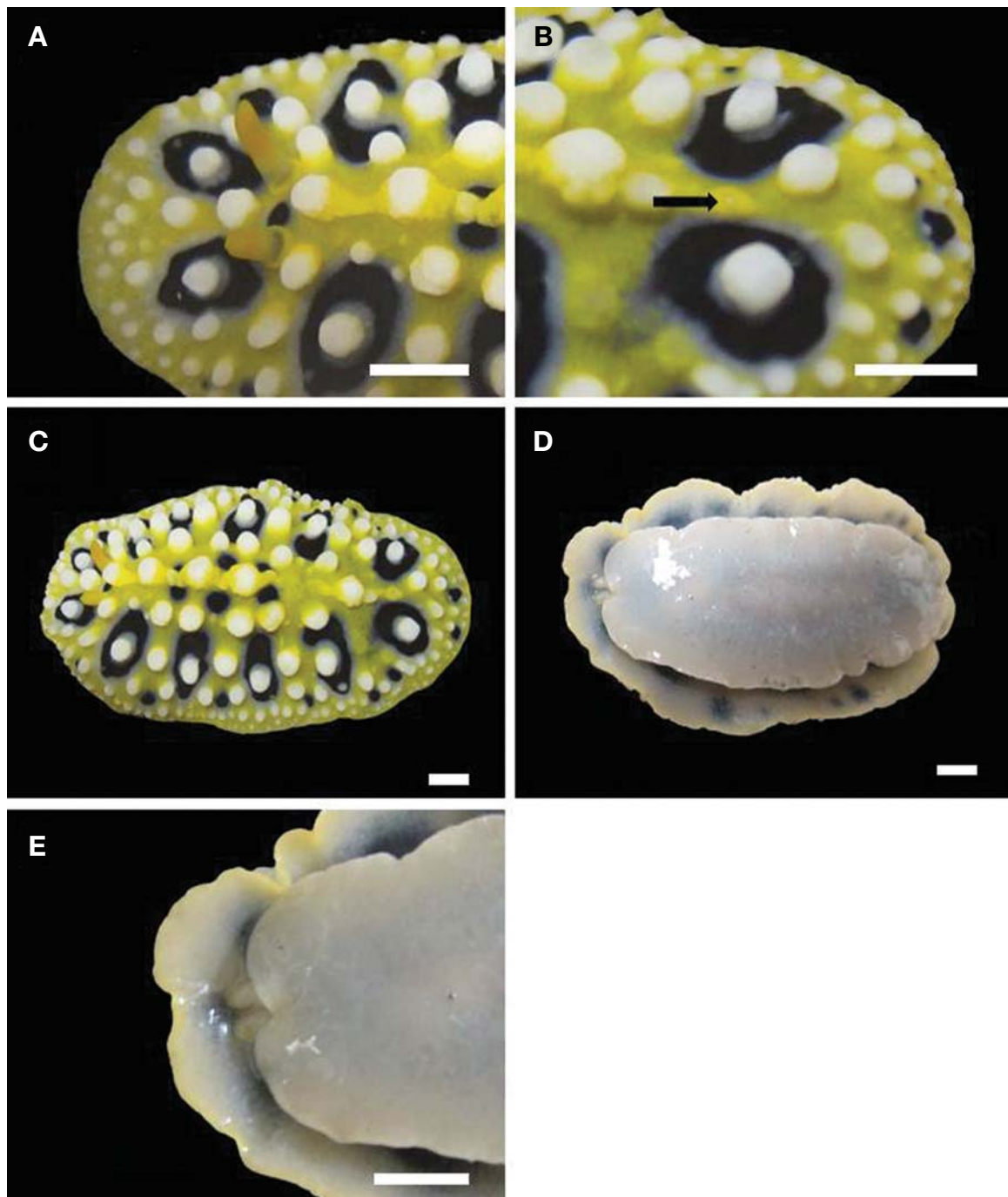


Fig. 1. *Phyllidia ocellata* Cuvier, 1804. A. rhinophores. B. anus. C. dorsal view. D. ventral view. E. oral tentacles. A-C. living animal. D, E. preserved specimen. An arrow indicates anus. Scales=5 mm.

Phyllidia verrucosa Hasselt, 1824: 244 (cited from Brunckhorst, 1993).

Phyllidia albonigra Quoy and Gaimard, 1832: 291-292, pl. 21, figs. 26, 27 (cited from Brunckhorst, 1993).

Phyllidiella nobilis Bergh, 1869: 485-492, 512-513, pl. 24b (cited from Brunckhorst, 1993).

Phyllidia spectabilis Collingwood, 1881: 136-137, pl. 10,

figs. 19-23 (cited from Brunckhorst, 1993).

Fryeria variabilis Collingwood, 1881: 137, pl. 10, figs. 24-28 (cited from Brunckhorst, 1993).

Phyllidia rotunda Eliot, 1904: 282-283 (cited from Brunckhorst, 1993).

Fryeria pustulosa: Haas, 1920: 140 (cited from Brunckhorst, 1993).

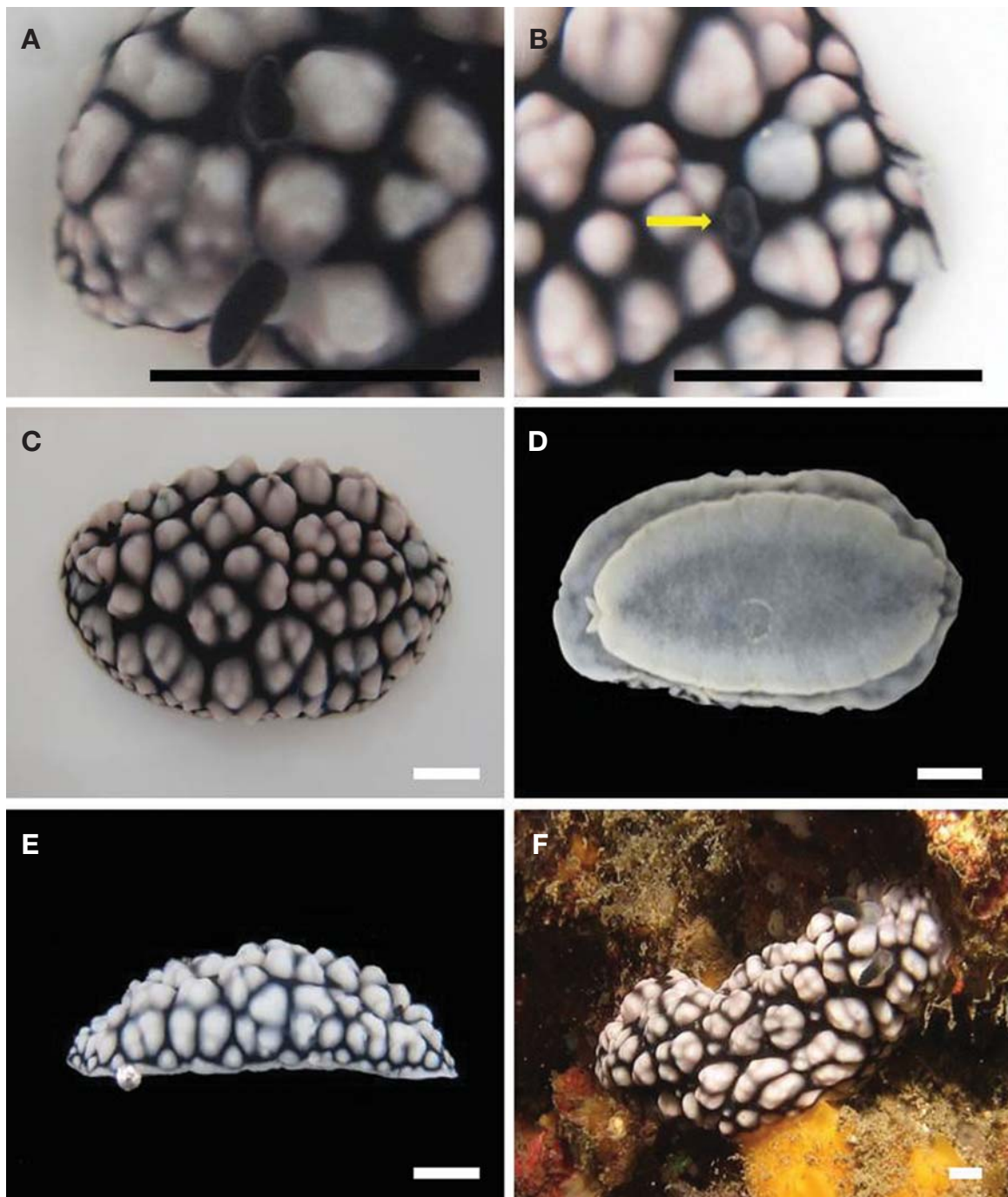


Fig. 2. *Phyllidiella pustulosa* (Cuvier, 1804). A. rhinophores. B. anus. C. dorsal view. D. ventral view. E. lateral view. F. habitus. An arrow indicates anus. A-E. preserved specimen. F. living animal. Scales=5 mm.

Phyllidia nobilis: Risbec, 1928: 58 (cited from Brunckhorst, 1993).

Phyllidia variabilis: Risbec, 1956: 24 (cited from Brunckhorst, 1993).

Phyllidia varians Pruvot-Fol, 1956: 68 (mis-spelling pro. *Fryeria variabilis* Collingwood, 1881) (cited from Brunckhorst, 1993).

Phyllidia melanocera Yonow, 1986: 1406-1407, figs. 2, 10f-i (cited from Brunckhorst, 1993).

Phyllidiella pustulosa: Brunckhorst, 1993: 49-54, figs. 3B, 9B-D, 11-13, 27, 28A-C. pl. 5E-F; Choe *et al.*, 1997: 39, figs. 4-5, tab. 3; Okutani, 2000: 795, fig. 2; Valdés, 2001: 339, figs. 1B, 5B-C, 6; Lee and Min, 2002: 145; Choi, 2003: 52, pl. 6; Debelius and Kuitert,

2007: 269; Gosliner *et al.*, 2008: 293.

Material examined. 1 individual (SMD0178), Jeju-do, Seogwipo-si, Seogwi-dong. 19 Feb 2012; 1 individual (KOSPIV0000164403), Jeju-do, Seogwipo-si, Seogwi-dong. 13 Oct 2013.

Diagnosis. Body ovate and elongate (length: 30-53 mm, width: 26-31 mm). Rhinophores lamellate and black in color (Fig. 2A). Anus open with black border (Fig. 2B). Back ground color black. Pinkish gray tubercles clustered 2-3 protuberances with variable in shape and size on dorsum (Fig. 2C, F). Sole of foot gray, divided anteriorly. Oral tentacles finger like in shape (Fig. 2D). Edge of mantle submarginal black line (Fig. 2E).

Distribution. Korea, Japan, Indo-west Pacific to Hawaii, New Caledonia, Fiji.

Family Polyceridae Alder and Hancock, 1845

Diagnosis. Varied body shape. Usually velar tentacles along anterior margin. Dorsal extrabranchial appendages and other lateral appendages (Gosliner *et al.*, 2008).

Genus *Thecacera* Fleming, 1828

***Thecacera pennigera* (Montagu, 1815) (Fig. 3)**

Doris pennigera Montagu, 1815: 17-18, pl. 4, fig. 5 (cited from Vallés *et al.*, 2000).

Thecacera pennigera: Fleming, 1828: 283 (cited from Willan, 1976); Alder and Hancock, 1855, fam. 1, pl. 21a, figs. 1-9 (cited from Baba, 1960); Willan, 1976: 347-352, fig. 1; Vallés *et al.*, 2000: 26, figs. 7C, 9; Debelius and Kuiter, 2007: 40.

Thecacera maculata Eliot, 1905: 241-243 (cited from Vallés *et al.*, 2000).

Thecacera pennigera var. *nigrescens* Labbé, 1931: 20 (cited from Willan, 1976).

Thecacera lamellate Barnard, 1933: 294-295, fig. 1 (cited from Vallés *et al.*, 2000).

Material examined. 1 individual, Gangwon-do, Goseong-gun, Jugwang-myeon, Munamjin-ri. 18 Aug 2012; 2 individuals (KOSPIV0000157372), Gyeongsangbuk-do, Uljin-gun, Wonnam-myeon, Doeksin-ri. 29 Aug 2012.

Diagnosis. Body slender (length: 13-17 mm, width: 9-12 mm) with translucent white. Blunte head. Pointed metapodium (Fig. 3A). Dorsum convex and high (Fig. 3B). Rhinophores lamellate with incomplete sheath. Rhinophores surrounded by bifurcate rhinophoral sheath (Fig. 3C). Gills five bipinnate on center of dorsum. Two very long elongated extrabranchial appendages on both sides of gills (Fig. 3D). Numerous yellow and black spots scattered (Fig. 3E, F).

Distribution. Korea, Japan, Parkistan, France, England,

Netherlands, Atlantic-USA, South Africa, Angola, Brazil, Australia, New Zealand.

DNA barcode. COI sequences of the first mentioned specimen in the "Material examined" are as follows: 5'-GTGCCTTTT TAGGGGACGATCATTTTATAATGTTATTGTTACTGCACATGCCTTTGTTATAATTTT TTTTCATAGTTATACCAGTAACCTATAGGAGGTTT CGGAAATTGAATAATTCCTTTATTAATTGGAGC TCCGGATATGAGTTTTCCTCGAATAAACAACAT AAGATTCTGATTTTACCTCCTTCATTTGTTTTA CTTTTATGTTCTACTCTCATGGAAGGGGGTGCTG GAACAGGTTGAACTGTTTACCCTCCTTTGTCTG GACCTGTAGGCCATAGGGGTGCTTCTGTGGATC TTGCTATTTTCTCTTTACATCTAGCAGGTGCTTC ATCATTATTGGCTTCAATTAACCTTTATTACTACT ATTCTTAATATACGGTCTTCAGCAATAAGTTTC GAGCGGGTGAGGTTGTTTGTATGGTCTCTTTTA GTGACAGCATTCCTTTTACTTCTTTCGTTACCGG TATTAGCAGGTGCTATTACTATATTACTGACA-3'.

According to BLAST search to GenBank, this sequence matches 86% with a COI record of *Thecacera pennigera* (AJ223277; reported from Spain), single COI record from this species in the GenBank. But, the similarity value shown between two records was not significant indication that two COI records were derived from the same species even though the identification of the species was correct. This means that this species is composed of at least two separate species. Therefore, to prove it more COI records are needed from diverse known localities.

Remarks. *Thecacera pennigera* is widely distributed worldwide. Willan (1976) suggested that the species spread beyond its original distributional range of distribution by transportation by ship. By examination of present specimens, there was no discrete morphological difference between present specimens and those from other countries except size and number of spots on mantle.

Genus *Triopha* Bergh, 1880

***Triopha catalinae* (Cooper, 1863) (Fig. 4)**

Triopha catalinae Cooper, 1863: 59.

Triopha carpenteri Stearns, 1873: 78, fig. 2 (cited from McDonald, 1983).

Triopha carpenteri: Bergh, 1880: 112-117 (cited from McDonald, 1983); MacFarland, 1966: 106, pls. 19, 29, 31; Okutani, 2000: 779, fig. 2.

Triopha modesta Bergh, 1880: 261-266, pl. 14, figs. 17-20 (cited from McDonald, 1983).

Triopha modesta: Fischer, 1887: 527 (cited from McDonald, 1983).

Triopha catalinae: Cockerell, 1915: 229 (cited from McDonald, 1983); Ferreira, 1977: 388-396, figs. 1-11, 16; McDonald, 1983: 215; Goddard, 1984: 153; Debelius

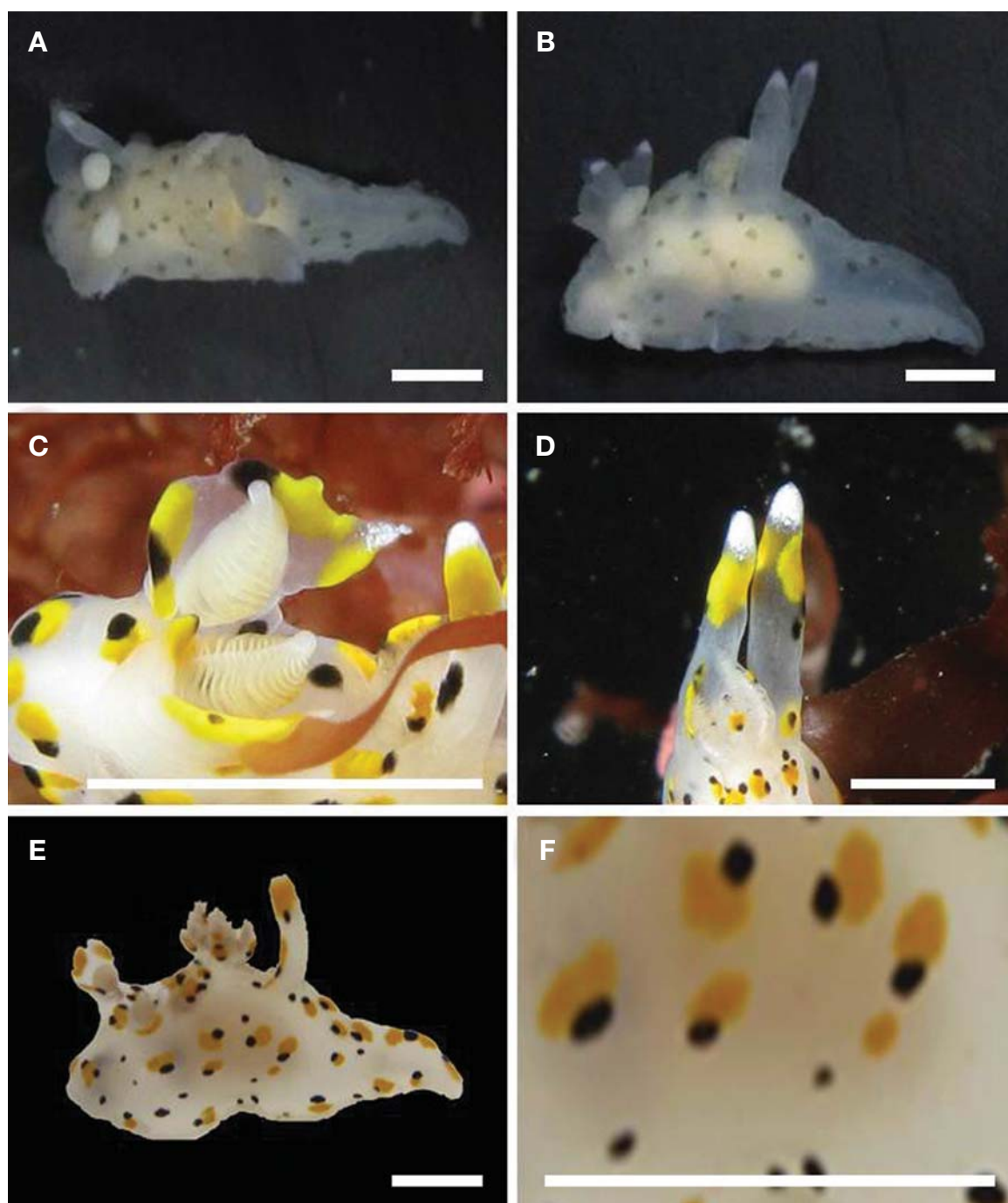


Fig. 3. *Thecacera pennigera* (Montagu, 1815). A. dorsal view. B. lateral view. C. rhinophores and rhinophoral sheath. D. gills and extra-branchia appendages. E. habitus, living animal. F. pattern of spots. A, B. preserved specimen. C-F. living animal. Scales=1 mm.

and Kuitert, 2007: 46; Gosliner *et al.*, 2008: 277.

Triopha scrippsiana Cockerell, 1915: 228-229 (cited from McDonald, 1983).

Triopha elioti O'Donoghue, 1921: 165-167 (cited from McDonald, 1983).

Material examined. 1 individual, Gangwon-do, Goseong-gun, Toseong-myeon, Bongpo-ri, 28 May 2012; 3 indi-

viduals, Gangwon-do, Yangyang-gun, Hyeonbuk-myeon, Gisamun-ri, 6 Jun 2012; 3 individuals (KOSP000016 5265), Gangwon-do, Goseong-gun, Jugwang-myeon, Munamjin-ri, 18 Aug 2012.

Diagnosis. Body elongate (length: 46-100 mm, width: 15-38 mm) and translucent pale white, Anterior rounded, Posterior pointed (Fig. 4A). Head flattened and expand-

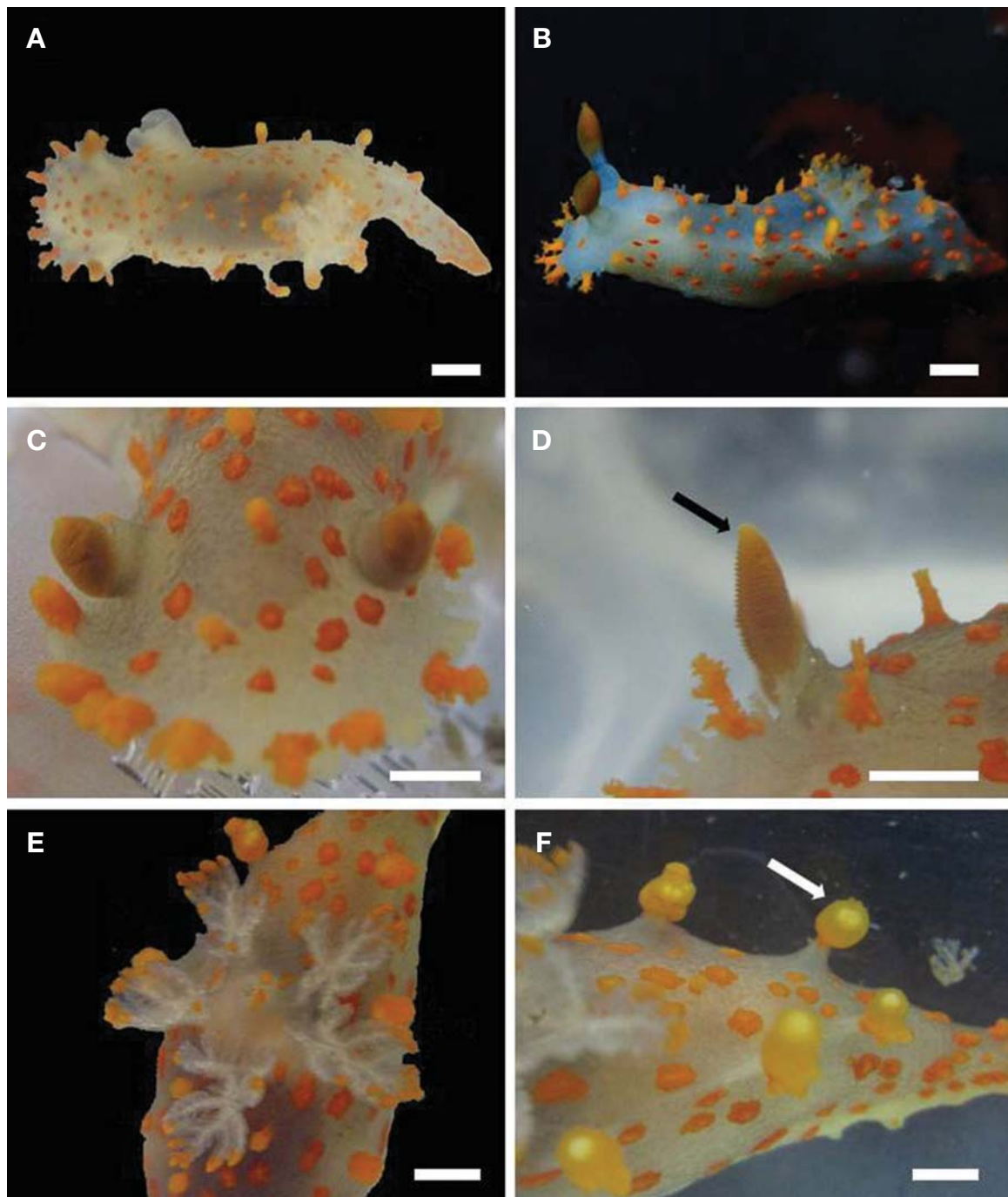


Fig. 4. *Triopha catalinae* (Cooper, 1863). A. dorsal view. B. lateral view. C. head. D. rhinophore. E. gills. F. tubercles. A-F. living animal. Scales=5 mm.

ed wider than body (Fig. 4B). Several margin process of frontal veil along anterior of head (Fig. 4C). Rhinophores lamellate and retractable (Fig. 4D). Gills five of simple tripinnate and non-retractable (Fig. 4E). Dorsum slightly arched, Usually several rounded end tubercles on edge of dorsum (Fig. 4E). Same orange color present tip of appendage: Rhinophores, Dorsal-lateral papillae, Dorsal

tubercles, Frontal veil margin process, Gill branch, Posterior end.

Distribution. Korea, Japan, Alaska, Baja California, Mexico.

DNA barcode. COI sequences of the first mentioned specimen in the “Material examined” are as follows: 5'-AGCTGGTGCATTTCTAGGGGATGATCATTTTAA

TAATGTCATTGTAACTGCTCATGCGTTCGTAAT
AATTTTTTTTATAGTTATGCCGTTAATAATCGGA
GGATTTGGTAACTGAATAGTTCCTTTACTAATT
GGAGCACCTGATATAAGTTTTCTCTCGAATAAAT
AATATAAGATTTTGACTTCTTCCCCCTCATTTA
TTTTATTGTTGTGTTCAACATTAATAGAAGGAG
GAGCTGGGACAGGATGAACGTGTGTACCCTCCTT
TATCTGGTCCTGTGGGTCATGGAGGTACGTCTG
TAGATCTTGCTATTTTTTCTCTCCATTTAGCTGG
CGCATCTTCTTTACTTGGGGCCATTAATTTTATT
ACTACTATTTTTAATATACGCTCTTCGGCTATAA
CTATAGAACGATTAAGTTTATTCGTTTGGTCTGT
TTTGGTGACTGCTTTTCTACTCTTGCTTCTTTA
CCTGTACTAGCCGGAGCTATTACTATACTAT-3'.

According to BLAST search to GenBank, this sequence matches 99% with a COI record of *Triopha catalinae* (GQ292040) available in the GenBank. This may support the morphological identification of the species.

Remarks. *Triopha catalinae* distributed from Alaska to Baja California in the Eastern Pacific. From the Western Pacific Okutani (2000) recorded this species from Sanriku and Hokkaido. The present report of *T. catalinae* from Korea showed a distributional extension of its previously known range.

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Front Cover

This cover shows the habitat of Korean nudibranch, *Phyllidiella pustulosa* (Cuvier, 1804). This article by Jung *et al.* can be found on pages 7-14. Photo by Daewui Jung.